Mechanisms of Spontaneous Human Cancers

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The causes of much of human cancer remain obscure. The fraction that is spontaneous is unknown and cannot be calculated until all known external causes have been accounted for. This is not a feasible proposition. However, there is substantial evidence that about 80% of human cancer could be avoided by eliminating tobacco consumption; by dietary changes; by reducing infection with certain viruses, bacteria, and parasitic worms; and, in white populations, by avoiding sunburn. Alcohol, occupational and medical carcinogens, and certain patterns of reproductive behavior also contribute to the cancer burden. Cancers that cannot be attributed to these causes, and for which no other causes can be found, could be considered spontaneous and to arise from endogenous processes. Epidemiological evidence suggests that spontaneous and induced cancers share the same mechanism. Cancer is a genetic disorder of somatic cells. An accumulation of mutant genes that control the cell cycle, maintain genomic stability, and mediate apoptosis is central to carcinogenesis. Spontaneous mutation may cause spontaneous cancer. Endogenous causes of mutation include depurination and depyrimidation of DNA; proofreading and mismatch errors during DNA replication; deamination of 5-methylcytosine to produce C to T base pair substitutions; and damage to DNA and its replication imposed by products of metabolism (notably oxidative damage caused by oxygen free radicals). Deficiencies in cellular defense mechanisms may also provoke spontaneous mutation. These include defective DNA excision–repair; low levels of antioxidants, antioxidant enzymes, and nucleophiles that trap DNA-reactive electrophiles; and enzymes that conjugate nucleophiles with DNA-damaging electrophiles. Mechanisms underlying many of these cellular defenses are under genetic control. Thus, germ line mutations or polymorphisms of genes that govern them may also contribute to spontaneous cancer. — Environ Health Perspect 104(Suppl 3):633–637 (1996)

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What Is Spontaneous Cancer?

Before addressing the mechanisms that might underlie spontaneous cancer, it would be useful to examine what such a term might mean. Spontaneous can be defined in several ways. For example, the *Shorter Oxford Dictionary* (*1*) provides several definitions, including "Of natural processes: having a self-contained cause or origin"; "growing or produced naturally without cultivation or labour"; and "produced, developed, or coming into existence by natural processes or changes." It is beyond dispute that much of human cancer is not spontaneous as defined above but is caused by exogenous agents or is self-inflicted by habits such as smoking and drinking alcoholic beverages or results from germ-line mutations in critical genes. (I shall not attempt to grapple with the question as to whether such mutations are themselves spontaneous, and the cancers that result are also spontaneous.) Such knowledge has been gained by studying differences in cancer incidence and mortality among different settled communities and between migrants and those who stay behind. Variation with time in cancer incidence and mortality within communities and the identification of specific causes or preventive factors have also contributed to our present knowledge of the causes of cancer (*2*). Moving from cause to mechanism has been made possible by remarkable advances in the molecular biology and genetics of cancer, and it is probable that the key events in carcinogenesis will be understood at the molecular level within the lifetime of a graduate student just embarking on a career in cancer research. Nevertheless, as things stand, the causes of much of human cancer remain obscure. The proportion that is truly spontaneous (and therefore unavoidable) is unknown and cannot be calculated unless and until all known external and genetic causes have been accounted for.

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Avoidable Cancer

There is substantial evidence that a major proportion (perhaps about 80%) of human cancer could be avoided by reducing or eliminating tobacco consumption; by changes in diet and nutrition; by reducing infection with certain viruses, bacteria, and parasitic worms; and, especially for white-skinned populations, by avoiding sunburn. Consumption of alcoholic beverages, certain aspects of sexual and reproductive behavior, and occupational and medical exposure to carcinogens also contribute to the cancer burden (Figure 1) (*2*). Poverty and wealth must also be taken into account when discussing the causes of cancer because there is impressive evidence that some cancers occur more frequently in poor communities and that others are more frequent among the rich (*3*) (see Figure 2 for some examples). Those cancers that cannot be attributed to these causes and for which no other causes can be found could be considered spontaneous and to arise from endogenous processes.

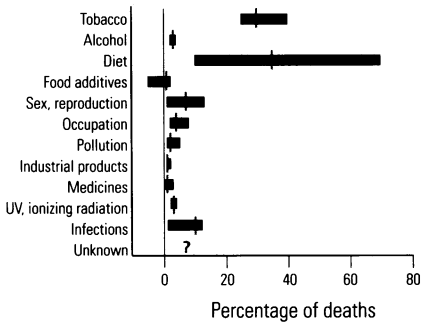

Figure 1. Proportions of cancer deaths attributed to various causes. Data from Doll and Peto (*2*). The vertical line on each bar represents the best point estimate and the bar itself represents the range of acceptable estimates.

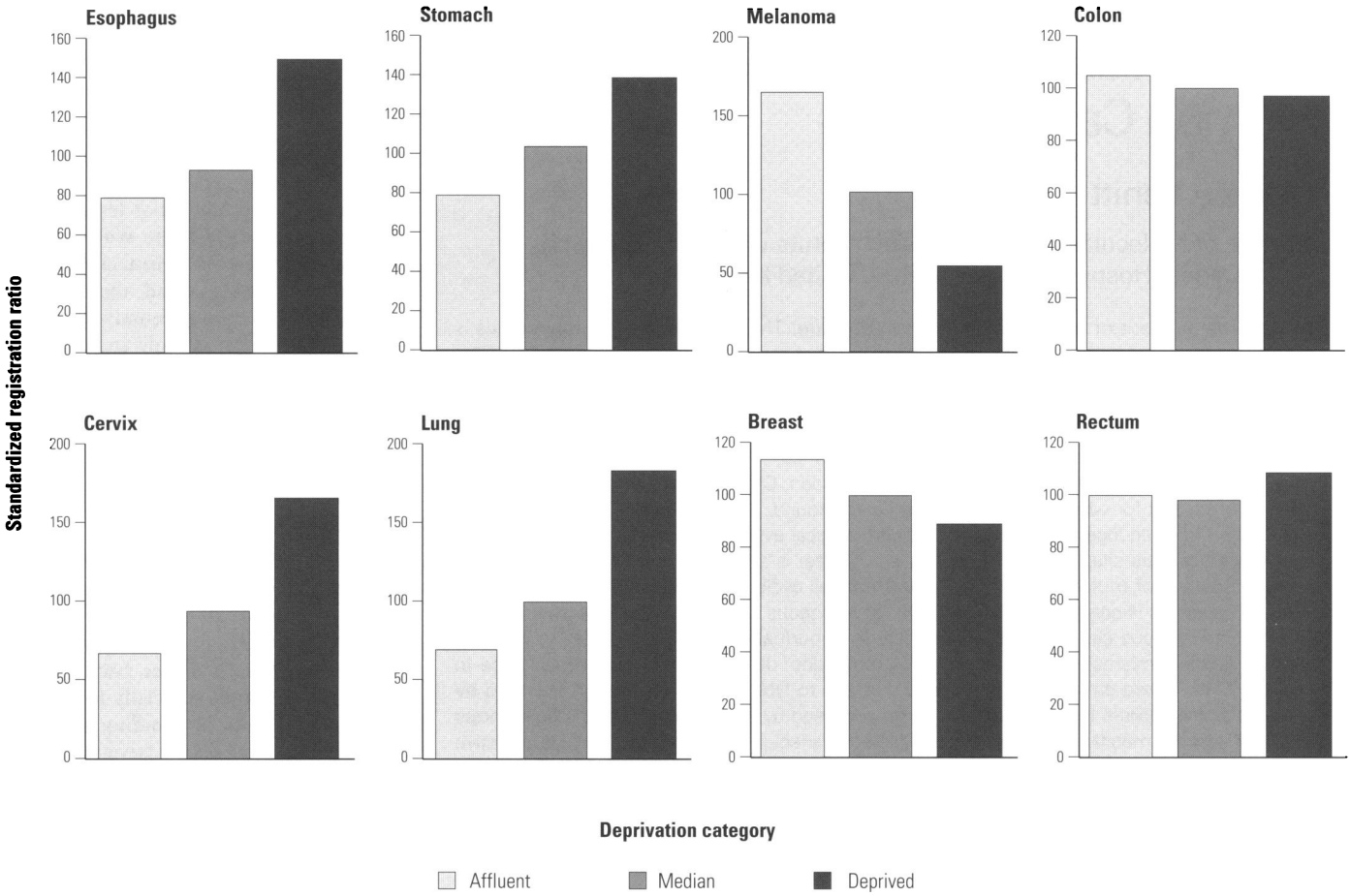

Figure 2. The relationship between deprivation category and incidence of eight different cancers in Scotland from 1979 to 1982. Data from Carstairs and Morris (*3*).

Nature, Nurture and Luck

With rare exceptions, development of cancer in an individual depends on a complex interplay among genetics, environment, and the play of chance—"nature, nurture and luck" and "what people do to themselves and what they have done to them" (*2*). It is therefore impossible, at present, to disentangle the relative contributions that each of these factors makes to the risk of cancer in an individual or within a given population. The problem has been addressed by Knudson (*4*). He suggested that the population can be divided into four oncodemes (an oncodeme is defined as a demographic unit with a peculiar sensitivity to a particular cancer), depending on the relative contributions of environment and genetics to the risk of cancer. These oncodemes are background (due to random mutations in normal people); environmental (due to environmental carcinogens [chemicals, radiation, or viruses, or combinations of these] in normal people); environmental/genetic (due to genetic susceptibility to environmental carcinogens); and genetic (genetic susceptibility is more important than spontaneous or environmentally induced events). Most human cancer probably occurs in the second and third oncodemes because exposure to environmental carcinogens is difficult or impossible to avoid and cancers that are due entirely to an inherited predisposition are known to be rare (*5*).

Do Spontaneous and Induced Cancers Share the Same Mechanism?

There is circumstantial evidence that the mechanisms that provoke what are thought of as spontaneous cancers appear to be the same as or similar to those that underlie cancers caused by exogenous agents. Figure 3 depicts one such piece of evidence, which is based on the analysis of the age distribution of lung cancer in smokers and nonsmokers by Doll (*6*). He showed that the excess rate of annual lung cancer incidence among regular smokers, which depends strongly on the duration of smoking, can be distinguished from the background rate derived from the incidence of lung cancer

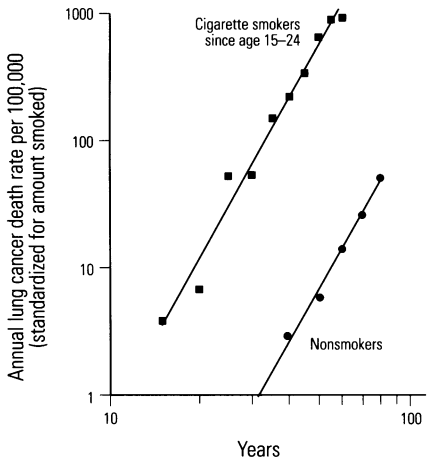

Figure 3. Lung cancer death rates in nonsmokers as a function of age and in regular cigarette smokers as a function of duration of smoking. Data from Doll (*6*).

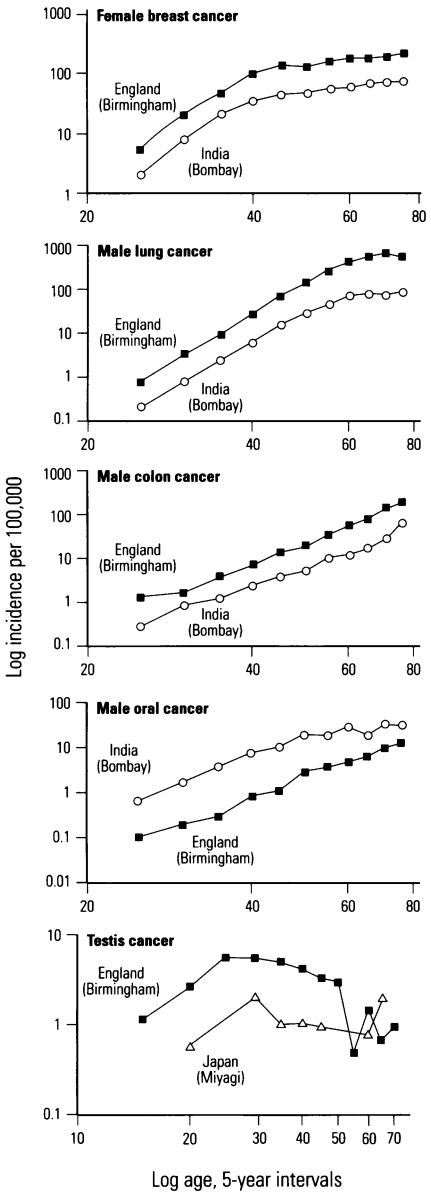

Figure 4. Log cancer incidence as a function of log age in high- and low-incidence countries. Data from the International Agency for Research on Cancer (*7*).

in nonsmokers. After adjustment for the effects of dose (numbers of cigarettes smoked), the slope (4.22) of the line obtained by plotting log lung cancer incidence for smokers as a function of log duration of smoking is remarkably similar to that obtained (4.17) by plotting log incidence of lung cancer as a function of log age in nonsmokers. Doll interpreted this as suggesting that "...non-smokers are exposed from birth to an agent that is capable of causing bronchial carcinoma (albeit a very weak one)...." This similarity of slopes could also be construed as indirect evidence that exogenous carcinogens—in this case tobacco smoke—enhance or intensify aberrant biological processes that occur spontaneously. Examination of the relationship between incidence and age in populations at high and low risk of particular cancers adds support for this contention, if we assume that the cancers in low-risk populations are more likely to be spontaneous than those in the high-risk population. Some examples of this approach are shown in Figure 4. In each case, the age–incidence curve in the low-incidence population runs parallel with that of the high-incidence population but is shifted to the right. This holds true for the four examples of epithelial cancers (breast, lung, colon, and oral cancer) and for testicular cancer in which the incidence declines after age 30. These comparisons do not prove that spontaneous cancer shares the same mechanism as cancers caused by tobacco smoke or oral tobacco or are related to reproductive history or diet; but these data do suggest that such exogenous factors enhance or accelerate a biological process that would occur anyway but at a much lower incidence and at older ages. Thus, if exogenous carcinogens do accelerate spontaneous carcinogenesis, it is likely that when all the preventable cancers have actually been prevented (an unlikely eventuality), the remaining cancers will appear at later ages and at much lower rates.

Cancer Is a Genetic Disorder of Somatic Cells

It is now generally accepted that cancer is a genetic disorder of somatic cells and that mutation of genes, whose products mediate signal transduction, control the cell cycle, maintain genomic stability, and mediate apoptosis and cellular senescence, is central to carcinogenesis. The number of mutations necessary to establish the full cancer phenotype is still under investigation and may vary from one type of cancer to another; this number of mutations is probably at least 2 and, in many types of cancer, not less than 5 or 6, as judged by interpretation of the age dependence of various human cancers [reviewed by Lawley (*8*)]. Direct evidence for the serial accumulation of independent mutations during carcinogenesis has been obtained from studies of the occurrence of mutant protooncogenes, tumor-suppressor genes, and mismatch-repair genes at successive histopathological stages that mark progression from normal tissue to a fully malignant tumor. The most well-documented and widely quoted example is colorectal cancer (*9,10*).

Spontaneous Cancer Arises from Spontaneous Mutation

Accepting that an accumulation of somatic mutations, however provoked, can initiate and sustain carcinogenesis, there will always be a background incidence for any given cancer because spontaneous mutation is inescapable, given the inherent chemical instability of DNA, the prodigiously large target that it presents to mutagens, the number of times it has to replicate, and the less-than-perfect defenses that have evolved to protect its integrity as a self-replicating carrier of information. It could be argued that cancer is indeed an inevitable by-product of evolution because mutation is the driving force of evolution, and without evolution there would be no life-forms for cancer to happen to. It is reasonable to propose, therefore, that spontaneous cancer arises from spontaneous mutation (oncodeme 1). Other factors could contribute; for example, a spontaneous increase in the rate of cell division might increase the probability of conversion of a endogenously generated promutagenic lesion into a mutation. The risk of sustaining a crucial mutation at a critical time in a crucial cell, in the absence of any exogenous cause or predisposing genetic background, will depend to some extent on chance (i.e., bad luck).

The Background Mutation Rate of Human Cells Is Very Low

Each cell division of a somatic diploid mammalian cell requires the accurate and timely distribution of 6×10^9 base pairs of DNA (2.2 m in length) to each daughter cell. In long-lived species like *Homo sapiens,* this process must operate accurately and unremittingly over many decades. Errors can arise if the replication machine selects the wrong nucleotides during polymerization of a new daughter strand using the template provided by the parental strand. Such misincorporation can result in mismatches. Several editing mechanisms prevent accumulation of coding errors during DNA replication, which is remarkably accurate, with error frequencies that range between 10^{-9} and 10^{-11} per base pair replicated (*11*). The background mutation rate is estimated to be about 1.4×10^{-10} mutations per base pair per cell generation, based on measurement of forward mutations in the hypoxanthine guanine phosphoribosyltransferase (*hprt*) gene in cultured human

lymphoid cells or electrophoretic protein variants at unselected loci (*12,13*). Loeb (*12,13*), in considering the role of mutation in carcinogenesis, has argued that this background mutation rate cannot account for more than two or three mutations (bearing in mind that cancer is a clonal disease originating from a single mutant cell) and cannot explain the numbers of mutant genes already known to occur in cancers, let alone the larger numbers that may be found in the future, even if clonal evolution is assumed to increase the selective pressure on mutations that confer a large growth advantage. He therefore suggested that a mutator phenotype that increases the rate of mutation is necessary to explain the number of serial mutations required for carcinogenesis. Such a phenotype would result from loss or impairment of mechanisms that prevent mutations arising during and after DNA replication. If this phenotype were to arise early in carcinogenesis, it would explain the genomic instability that is characteristic of human cancers and the number of mutant genes observed in them. Mutator phenotypes and their corresponding genotypes have been discovered in the human germ line and in human cancers, suggesting indeed that genomic instability provides a powerful drive to carcinogenesis. Examples of such germ-line mutations include those in genes underlying rare recessively inherited disorders such as ataxia telangiectasia (*14*) and more common, dominantly inherited genes such as *MSH2, hMLH1, hPMS1* and *hPMS2*—mismatch-repair genes that predispose to hereditary nonpolyposis colorectal cancer (HNPCC) (*15*). The role of mutator phenotypes in spontaneous cancer is unknown, but there is no reason to exclude them from consideration in that context.

Endogenous Processes that Lead to Mutation

Lindahl (*16*) has reviewed the various chemical and enzymatic processes that may account for spontaneous mutation. These include spontaneous depurination and depyrimidation of DNA in the aqueous milieu of the cell, mismatch and proofreading errors during DNA replication, and deamination of 5-methylcytosine at CpG dinucleotides to produce C to T base pair substitutions (see Table 1).

Deamination of 5-Methylcytosine Acts as an Endogenous Mutagen

In about 99% of the mammalian genome, CpG dinucleotides are underrepresented by about one-quarter of the proportion expected from the observed frequency of C and G in the DNA. There is a corresponding overabundance of TpG and CpA dinucleotides. This depletion of CpG dinucleotides in mammalian genomes is thought to result from the readiness of 5-methylcytosine (5mC) to undergo oxidative deamination at the exocyclic amino group to form thymine, which, being a normal base, is not readily recognized by repair enzymes; this results in mutations, in particular, the 5mC → T transition (CpG → TpG). Despite the rarity of CpG dinucleotides and the existence of a G–T mismatch repair system, this transition occurs about 10 times as frequently as other transitions and is frequently seen in inherited diseases—over 35% of point mutations leading to human genetic disease are CpG → TpG transitions (*11*). Thus, 5mCpG sites are particularly prone to loss by mutation, and 5mC can be thought of as an endogenous mutagen. CpG → TpG transitions are also common in the p53 gene in human cancers. It is estimated that about half of all cancers that occur in the United Kingdom and the United States contain p53 mutations (*17–19*). Mutations, mainly of the missense type (transitions and transversions), are scattered over a wide area of the gene but occur most frequently in those regions most highly conserved during evolution, particularly exons 5 to 8. At least four hotspots for mutation can be identified, the frequency and distribution of which vary among different kinds of cancer. Transitions at the CpG dinucleotide occur frequently in p53 in many cancers and probably reflect endogenous mutation. A reduction in the proportion of CpG mutations at the expense, for example, of an increase in G → T transversions may signal the effect of an exogenous mutagen. An example is shown in Figure 5. In the germ line and in colorectal cancer, the majority of p53 mutations are G → A and C → T transitions that occur predominantly at CpG dinucleotides (*17,19*). This spectrum is very similar to that seen in the germ line in an unrelated gene, hemophilia B, and is

Table 1. DNA instability, damage, and defense mechanisms involved in spontaneous mutation.

Event	Example of damage	Defense
DNA depurination	Apurinic site	AP endonuclease + error-free repair
Deamination of cytosine	Replacement of C by U	Uracil glycosylase + error-free repair
Deamination of 5-methylcytosine to thymine	Replacement of C by T	Mismatch repair Evolution to deplete genome of CpGs
Alkylation of guanine and adenine	7-Methylguanine, 3-methyladenine	3MeA-glycosylase + error-free repair Spontaneous loss of 7MeG
Oxidative damage	8-Hydroxyguanine	Specific glycosylase + error-free repair; Antioxidants; catalase, superoxide dismutase

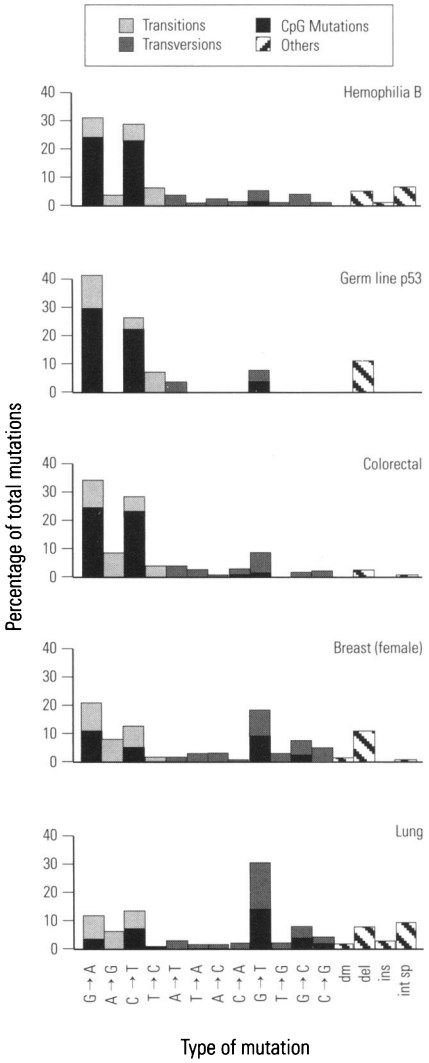

Figure 5. Spectrum of somatic mutations in exons 5 to 8 of p53 in the colorectum, female breast, lung, germ line, and hemophilia B gene. Abbreviations: dm, double mutant; del, deletion; ins, insertion; int sp, intron splice. Redrawn from Biggs (*17*).

thought to represent mutations arising spontaneously. In lung cancer, however, there is a marked reduction in the proportion of such mutations, concomitant with an increase in the proportion of G → T transversions, such that these predominate. This is consistent with the action of exogenous carcinogens present in tobacco smoke, which cause G → T transversions. Breast cancer is an interesting case: the spectrum of mutations is intermediate between that for colorectal cancer and that for lung cancer. This implies that an environmental agent or agents could be responsible for a proportion of human breast cancer (*17*). Given that deamination of 5-methylcytosine to thymine appears to be a powerful source of endogenous mutation, it is interesting to note that all 46 CpG sites on both strands in exons 5 to 8 of the p53 gene are methylated and that methylation is independent of tissue type (*20*).

Oxidative Damage, Defects in Detoxification, and DNA Repair

Other events that could produce spontaneous mutations include insults to DNA and its replication imposed by products of metabolism, the most notable being oxidative DNA damage caused by oxygen free radicals produced by normal aerobic metabolism (*16,21*). Deficiencies in those mechanisms that have evolved to protect cells against genetic accidents may also contribute to spontaneous mutation. These include defective DNA excision–repair, low levels of antioxidants and antioxidant enzymes (e.g., superoxide dismutase), low levels of nucleophiles (e.g., glutathione) that trap DNA-reactive electrophiles, and defects in enzymes (e.g., glutathione *S*-transferase) that conjugate nucleophiles with DNA-damaging electrophiles (*16*). Suboptimal concentrations of substrates (e.g., *S*-adenosylmethionine) that are involved in biomethylation of DNA might also contribute to spontaneous mutation (*22*). The mechanisms that operate in these three categories are all under genetic control. Thus, germ-line mutations or polymorphisms of genes that govern them may also contribute to spontaneous cancer.

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